American Journal of Hematology 58:36–41 (1998)

Two Distinct Truncated Variants of Ankyrin Associated With Hereditary Spherocytosis

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We present two distinct truncated variants of ankyrin associated with mild to moderate hereditary spherocytosis. Ankyrin Saint-Etienne 1 was manifested by an additional band located between bands 2.1 and 2.2. It was associated with a nonsense mutation in exon 39: TGG→TGA; W1721X. Ankyrin Saint-Etienne 2 appeared as two faint bands underlining bands 2.1 and 2.2. It was associated with a nonsense mutation in exon 41: CGA→TGA; R1833X. Overall ankyrin was diminished in splenectomized patients. Messenger RNAs Saint-Etienne 1 and 2 amounted to 20 and 37% of the total ankyrin mRNA, respectively. Ankyrin molecules truncated in their *C*-terminal region retain some ability to bind to the membrane whereas the bulk of nonsense mutations, located in more upstream regions, result in the mere disappearance of one haploid set of ankyrin. In the present cases, it was not possible to apportion the roles of ankyrin reduction and truncation in the pathogenesis of hereditary spherocytosis. Am. J. Hematol. 58:36–41, 1998.

Key words: hereditary spherocytosis; *ANK1* gene; nonsense mutations; truncated ankyrins

INTRODUCTION

Erythroid ankyrin is encoded by the *ANK1* gene, a 42 exon gene [1]. It has numerous isoforms. Band 2.1 (apparent MW:210 kDa) contains all the exons. Band 2.2 (apparent MW:195 kDa) results from a mRNA spliceoform in which the 5′ part of exon 38 has been spliced out [1]. The mechanisms that generate shorter bands 2.3 and 2.6 have not been deciphered with certainty. There are hints that they arise from proteolysis, resulting from cleavages in more inward regions of ankyrin [2,3]. A number of minor mRNA spliceoforms with no detectable polypeptide counterpart have been described [1]; however, their biological significance is not established. Of note, reticulocytes are richer in ankyrin (mainly understood as bands 2.1 and 2.2) than mature red cells so that ankyrin deficiency may be masked by a high reticulocyte count. Following splenectomy, reticulocytes fall off and ankyrin deficiency becomes more evident. Mutations in the *ANK1* gene are the most common cause of hereditary spherocytosis (HS) [4–9; for review, see 10]. Frequent nonsense or frameshift mutations result in the premature termination of translation, if not in the mere absence of the mutated ankyrin mRNA [11]. One haploid set of an-

Contract grant sponsor: Centre National de la Recherche Scientifique; Contract grant number: CNRS URA 1171; Contract grant sponsor: Programme Hospitalier de Recherche Clinique; Contract grant sponsor: Université Claude-Bernard Lyon-I; Contract grant sponsor: Institut National de la Santé et de la Recherche Médicale; Contract grant number: CRE 930405; Contract grant sponsor: Conseil de la Région Rhône-Alpes; Contract grant sponsor: Fondation pour la Recherche Medicale; Contract grant sponsor: Association de Recherche contre le Cancer; Contract grant sponsor: Institut Pasteur de Lyon.

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Received for publication 28 May 1997; Accepted 10 December 1997

TABLE I. Hematological Data, Variant mRNA, and Protein Quantitation*

	Hb g/L	Reticulocytes $\times 10^9$/L	Pink test after 24 hr[a]	Ankyrin/band3 (% of normal)	mRNA variant (% of total ankyrin mRNA)	Ankyrin variant (% of total ankyrin)
Ankyrin Saint-Etienne 1						
Family A						
I.1 Proband's father	158	nd	1	96	—	—
I.2 Proband's mother	142	nd	2	94	—	—
II.1 ***Mother*** (proband)	112	410	36	107	19.8	17.0
II.2 Husband	147	nd	1	99	—	—
III.2 ***Son***	139	nd	10	98	21.6	17.5
Ankyrin Saint-Etienne 2						
Family B1						
I.1 ***Mother*** (proband)[b]	119	10	13	74		19.0
II.1 ***Daugher***[c]	115	201	35	95	37.6	17.0
II.1' Son[c]	130	5	2	99	—	
II.1'' ***Son***[c]	115	130	28	106	34.9	19.0
Family B2 (one individual)						
I ***Male*** (proband)[b]	173	92	29	71	38.6	19.0

*Bold italic characters: HS patients. b: triplet children. nd: not determined.
[a]Normal values: 2.7 ± 1.2% (n = 24).
[b]Splenectomized.
[c]Triplet children.

kyrin is missing in the membrane, the normal allele in *trans* achieving only a partial compensation. Spectrin [12] and protein 4.2 [3] are decreased in a secondary fashion. The inheritance pattern is usually dominant. Recent reports have shown that the recessive mode of transmission reflects, in fact, the occurrence of de novo mutations [5,8,9]. Only in one instance (dominant HS) has a weakly expressed, truncated ankyrin been recognized [13,14].

We report here two shortened variants of ankyrin in three unrelated families with HS. The condition was mild to moderate. These variants appeared as faint additional band(s) associated with a reduction of the overall ankyrin content, at least in splenectomized patients.

CASE REPORTS

Routine laboratory data are presented in Table I.

Ankyrin Saint-Etienne 1

Family A. The proband II.1 (Table I) had a sclerotic subicterus since childhood. A splenomegaly and a hepatomegaly were observed at the age of 28. She had a hemolytic crisis (Hb: 77 g/L) when she was 33. Her parents I.1 and I.2 were hematologically normal. The disease was also found in child III.2 on the occasion of a family survey. This child has not manifested any noticeable symptom so far.

Ankyrin Saint-Etienne 2

Family B1. The mother (I.1), splenectomized when she was 14 transmitted ankyrin Saint-Etienne 2 to two of her triplet children; the mother was 26 when they were born, after a 29-week pregnancy. Prematurity required transfusion. None of the newborn had hyperhemolysis. At the age of 5, HS child II.1 displayed a typical parvovirus aplastic crisis. She presently exhibits no pallor, nor jaundice. A spleen tip was noted. The development of the child II.1′′ has been normal up to now. The father was unavailable.

Family B2 (one person). This individual case has briefly been presented elsewhere [15]. The diagnosis of HS was established on the occasion of extramedullary hematopoiesis (mediastinal pseudotumors, compression of the inferior vena cava) that was surgically treated. Splenectomy was performed at the age of 68. We had no access to the family members.

METHODS

Most techniques dealing with osmotic resistance, protein chemistry, molecular genetics, and paternity tests have been presented in detail or quoted in previous articles [5,8,9]. In particular, SDS-PAGE was carried out according to Fairbanks et al. [16]. Total ankyrin refers to the sum of the following bands: 2.1 + 2.1′ + 2.2 + 2.2′ (if present) obtained following densitometric scanning (the prime designates a shortened band). In addition to the anti-ankyrin polyclonal antiserum prepared by ourselves, we used two other polyclonal antiserums (one kindly provided by Dr. D. Dhermy, and the other purchased from the Charles River Company (formerly East Acres Biologicals, Southbridge, MA).

Exons 34 to 42 were PCR-amplified. Specifically, we used the following primers on the basis of intronic sequences kindly provided by Pr. B.G. Forget prior to pub-

lication [1]: (1) exon 39: primer A (sense), $^{-26}$cagctgct-gcttcttctgga and B (antisense), $^{+37}$aaggaacagcagcacgcag; (2) exon 41: primer C (sense), $^{-77}$ccaaagtgctgagattacaga and D (antisense), $^{+18}$ccgcgggcgcctcagta. Search for single-strand conformation polymorphisms (SSCP), asymmetric PCR, RT-PCR, molecular cloning, nucleotide sequencing, restriction analysis, and determination of the $(AC)_n$ repeats located in the 3′-part of *ANK1* gene were basically done as described before [5,8,9]. Regarding quantitation of ankyrin Saint-Etienne 1 and 2 mRNAs based on RT-PCR, we chose 25 and 27 cycles, respectively, because they were in the interval of proportionality (not shown). For ankyrin Saint-Etienne 1, the PCR primers were: E (sense, exon 37), ^{4556}GCAACTT-GAAGCCAGACAG and F (antisense, exon 39), ^{5268}ACCTTGCGTGACCTCCTC, so that the alternatively spliced out 5′ part of exon 38 (spliceoform 2.2 or 2.2′) would be encompassed. For ankyrin Saint-Etienne 2, primers were: G (sense, exon 40), ^{5508}GCAGGT-GACAGAGGAGCAA and H (antisense, exon 41), ^{5666}AGCTCACTGGGATCCTCCA. Normal and mutant cDNA fragments were distinguished upon restriction analysis, since mutations Saint-Etienne 1 and 2 abolished a *Scrf*I site and a *Taq*I site, respectively. For ankyrin Saint-Etienne 1, *Scrf*I restriction analysis was also performed on purified RT-PCR fragments (primers E/F) corresponding to the cDNAs 2.2 and 2.2′. Quantitation was obtained by densitometric integration of the ethidium bromide-stained appropriate bands following gel electrophoresis.

RESULTS

Ankyrin Saint-Etienne 1

Family A. Unsplenectomized HS members disclosed normal amounts of ankyrin, due to a high reticulocyte count, at least measured in member II.1 (Table I). The amount of spectrin was in the lower part of the normal range and protein 4.2 was significantly ($P < 0.05$) decrease (not shown). These changes are typical of an ankyrin deficiency masked by an increase of the reticulocyte count. A faint additional band (apparent molecular weight: approximately 200 kDa) appeared between bands 2.1 and 2.2 (Table I, Fig. 1). Surprisingly, it did not react with any of the antiserums used (in contrast with the shortened bands defining ankyrin Saint-Etienne 2, see below). We speculate that these antiserums are directed against an epitope located between the mutations, e.g., *C*-termini of band 2.1′ of Saint-Etienne 1 and band 2.2′ of Saint-Etienne 2 (see below). Nevertheless, we assumed that the additional band was a shortened form of band 2.1. It was referred to as band 2.1′. The 2.2′ counterpart of band 2.2 was undetectable upon Coomassie blue staining, but small amounts of cDNA 2.2′ were present (see below). Bands 2.3 and 2.6, which are thought to lack larger *C*-terminal fragments, were not duplicated.

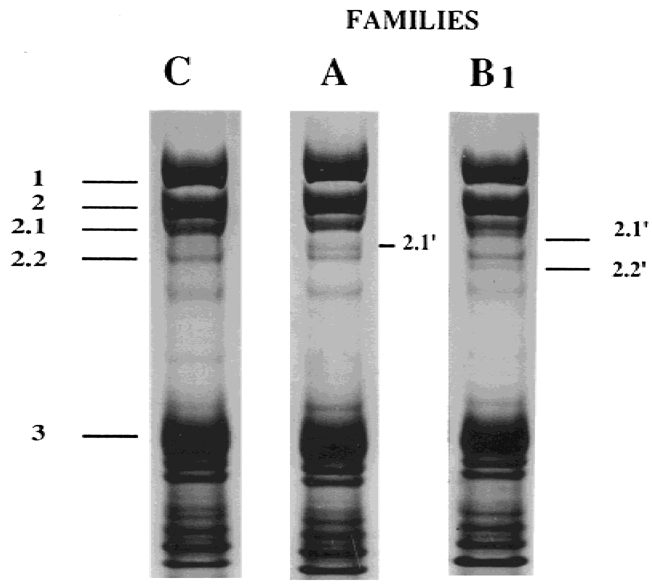

Fig. 1. Protein patterns on SDS-PAGE [14]. Lanes C: **Control;** A: **Individual III.2 from family A. Band 2.1′ corresponds to ankyrin Saint Etienne 1.** Lane B_1: **Individual II.1 from family B1. Bands 2.1′ and 2.2′ correspond to ankyrin Saint-Etienne 2.**

We examined only exons 34–42 because we assumed that the most likely, yet not the only way, for a shortened protein to be still viable is to carry a mutation removing its *C*-terminal region. This situation has been repeatedly encountered as regards spectrin β-chain in hereditary elliptocytosis [for review, see 17]. In addition, ankyrin isoforms 2.3 and 2.6 failed to exhibit a truncated counterpart, suggesting that the mutations would lie farther downstream of their *C*-termini.

We found a nonsense mutation in exon 39: TGG→TGA; W1721X in the two HS members (not shown). This mutation abolished a *Scrf*I site. Restriction analysis following PCR amplification of exon 39 (primers A/B) confirmed this fact (Fig. 2). Because primers E and F encompassed the alternative spliced out 5′ part of exon 38, RT-PCR followed by *Scrf*I digestion allowed separate analysis of cDNAs 2.1 + 2.1′, and cDNAs 2.2 + 2.2′. cDNA 2.1′ yielded 373, *234* and 106 bp fragments (vs. 373, 208, and 106 bp fragments in the control, the 26 bp fragment being undetectable) (Fig. 3). cDNA 2.2′ generated a *227* bp fragment (vs. the 201 bp 2.2 fragments in the control, the 26 bp fragment being undetectable) (Fig. 3). From quantitation of appropriate fragments, mRNA Saint-Etienne 1 amounted 20.7% on the average of total ankyrin mRNA (Table I). Spliceoform 2.2′ represented 20% on the average of the mRNAs 2.2 + 2.2′ amount (not shown). A schematic diagram of ankyrin Saint-Etienne 1 *C*-terminal region is provided (Fig. 4).

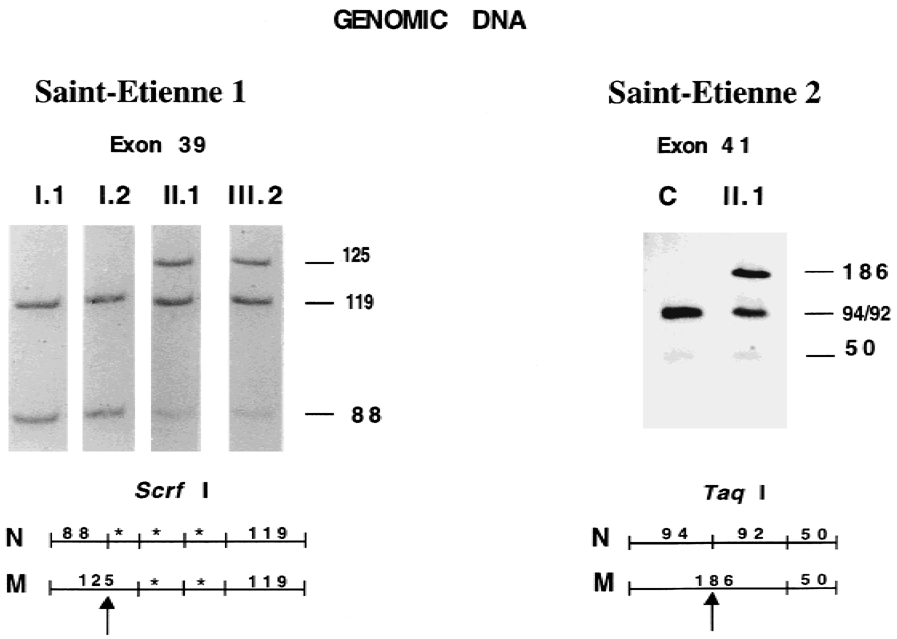

Fig. 2. Genomic DNA restriction analysis. Fragment sizes are given as nucleotides. Lane C: **Control. N and M: normal and mutant restriction maps. |: *Scrf*I or *Taq*I sites. ↑: abolished sites. Small *Scrf*I fragments (*) were not detected.**

Paternity tests failed to exclude false paternity with an error risk close to 1/3,300,000 between generations I and II. Most likely, mutation Saint-Etienne 1 occurred de novo on the $(AC)_{14}$ allele from individual I.1.

Ankyrin Saint-Etienne 2

Families B1 and B2. Splenectomized patients heterozygous for ankyrin Saint-Etienne 2 exhibited a 26–29% reduction of the overall ankyrin (Table I, Fig. 1). The spectrin and protein 4.2 amount were significantly ($P <$ 0.05) decreased (not shown). In unsplenectomized children II.1 and II.1′′ the increased reticulocyte count masked the ankyrin deficiency. Bands 2.1 and 2.2 were underlined by faint bands 2.1′ and 2.2′ (apparent molecular weights: approximately 205 and 189 kDa, respectively). They reacted with the three anti-ankyrin antiserums tested. Bands 2.3 and 2.6 were not duplicated.

A nonsense mutation was found in exon 41: CGA→TGA; R1833X, in all HS members from families B1 and in HS individual B2 (not shown). This mutation abolished a *Taq*I site. Using PCR amplification of exon 41 (primers C/D), *Taq*I digestion confirmed this fact (Fig. 2). Following RT-PCR (primers G/H), *Taq*I digestion of cDNA Saint Etienne 2 gave rise to a 159 bp fragment (vs. 87 and 72 bp fragments in controls) (Fig. 3). Spliceoforms 2.1 and 2.1′, and 2.2 and 2.2′ were not discernable owing to the positions of primers G and H. From the quantitation of appropriate fragments, we deduced that mRNAs Saint-Etienne 2 amounted to about 37% of total ankyrin mRNA (Table I). Mutation Saint-Etienne 2 was in *cis* of a $(AC)_{11}$ repeat in both families (not shown).

Direct nucleotide sequencing (primers not shown) of exon 34 showed a previously unreported polymorphism: TAC→CAC; Y1386C in all triplet children but not in their mother (family B1). This polymorphism was probably inherited from the unavailable father.

DISCUSSION

Ankyrin Saint-Etienne 1 and 2 (Fig. 4) are the second and third examples of truncated ankyrins described after ankyrin Prague [13,14], the genomic mutation of which has not yet been presented to the best of our knowledge. Ankyrin Rakovnik [18] achieved a different situation: a nonsense mutation in exon 38 was associated with the lack of band 2.1, whereas band 2.2 appeared normal in size and amount. The nonsense mutation was removed by the physiological alternative splicing of the 5′ part of exon 38 [1]. Ankyrin Saint-Etienne 2 reacted with the three antibodies used while ankyrin Saint-Etienne 1 did not. Since we do not know the involved epitope, we may only speculate that a strong epitope lies between the *C*-termini of ankyrins Saint-Etienne 1 (band 2.1′) and 2 (band 2.2′). Shorter bands 2.3 and 2.6 did not react since they do not carry this putative epitope, being much shorter than ankyrin Saint-Etienne 2 (band 2.2′).

Nonsense mutations often lead to instability and degradation of mRNAs [for review, see 19]. The more downstream the mutation, the less unstable the mutated mRNA. Although this general statement suffers exceptions, our data and those obtained by other authors [18] are in good agreement with it. The nonsense mutations in exons 39 and 41 reported here partially spared mRNAs 2.1′ whereas a nonsense mutation in exon 38 [18] resulted in the loss of mRNA 2.1. Specifically, the non-

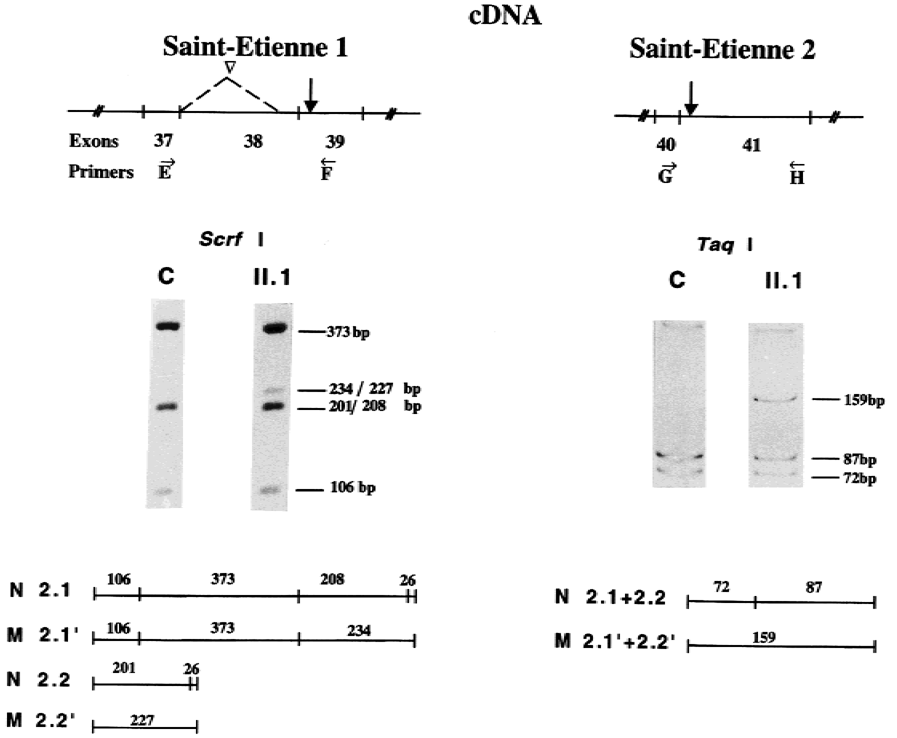

Fig. 3. Estimation of the variant mRNA amount. cDNA amplification products were digested, electrophoresed, stained, and scanned (see text). Left: **Saint Etienne 1. ▽: alternative spliced out fragments (mRNAs 2.2 and 2.2′). ↓: TGG→TGA mutation. *Scrf*I digestion products are shown in a patient (II.1) and a control (C) (detailed in the text).** Right: **Saint Etienne 2 cDNA analysis. ↓: CGA→TGA mutation. *Taq*I digestion products are shown in a patient (II.1) and a control (C) (detailed in the text).**

sense mutation in exon 39 spared mRNAs 2.1′ and 2.2′ as shown by RT-PCR (why band 2.2′ failed to appear on gels will be discussed below). The nonsense mutation in exon 41 spared mRNAs 2.1′ and 2.2′, which were undiscernable upon RT-PCR but generated isoforms 2.1′ and 2.2′ on gels. We had no way to apportion the roles of ankyrin reduction and truncation in the pathogenesis of HS.

RT-PCR results indicated that nonsense mutations Saint-Etienne 1 and 2 did not elicit the skipping of exons 39 or 41, respectively, as has been described in some cases (not concerning the *ANK1* transcript) [20,21].

Exons 41 and 42 lie in the regulatory domain of ankyrin [22]. Missing sequences may influence, through long distance conformational changes, the binding of ankyrin to band 3 [23]. Band 2.2′ of ankyrin Saint-Etienne 1 would lack parts of exons 38 and 39, and entire exons 40, 41, and 42. This truncation would be the largest described in this study. It would affect more strongly the stability and/or binding of ankyrin Saint-Etienne 1 to band 3 and account for its undetectability upon Coomassie blue staining (in spite of the presence of low amounts of the mRNA 2.2′). We could not perform in vitro tests likely to explore whether ankyrin Saint-Etienne 1 and 2 truncated isoforms had a more or less impaired binding to band 3.

It remains unexplained why the proportion of ankyrin Saint-Etienne 2 (bands 2.1′ and 2.2′) in the membrane was lower than that expected from the amount of the corresponding mRNAs. In both abnormal ankyrins,

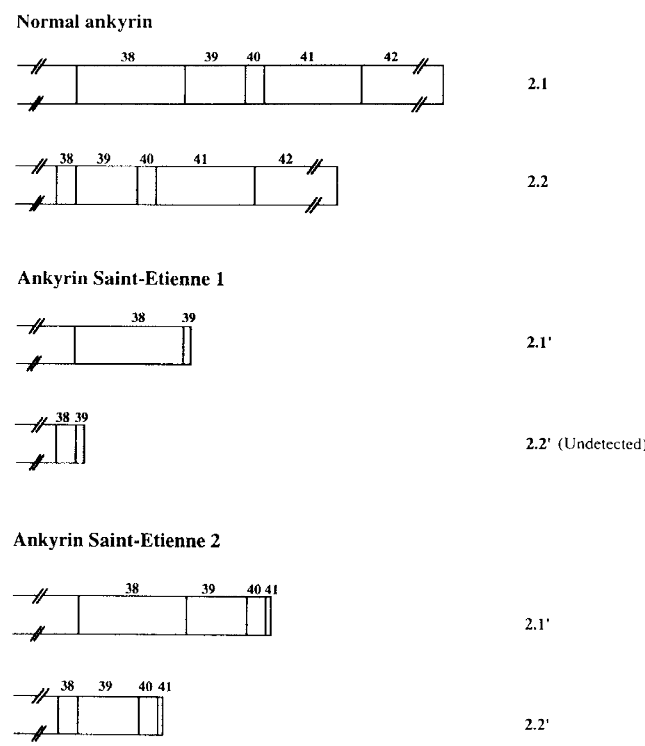

Fig. 4. Diagram depicting the *C*-terminal region of normal ankyrin and ankyrin Saint-Etienne 1 and 2. Band 2.1 isoform is the major isoform of ankyrin and contains the amino acids encoded by all the exons. Band 2.2 derives from a spliceoform lacking part of exon 38. Bands 2.3 and 2.6 are not represented because their *C*-termini lie upstream from the region shown. In ankyrin Saint-Etienne 1, isoform 2.2′ was not detectable (Comassie blue staining and Western blotting).

bands 2.3 and 2.6 were not duplicated, for their *C*-termini lie upstream from the nonsense mutations.

The clinical pictures exhibited some dissimilarities. Member I.1 (family B1) was splenectomized at a much younger age than individual I (family B2), both carrying ankyrin Saint-Etienne 2. The reason for these differences are ill-understood. Family A represents still another case in which the occurrence of a de novo mutation was observed in the *ANK1* gene.

To conclude, the observation of a truncated variant of ankyrin reasonably directs the search for the responsible mutation toward the 3′-region of the *ANK1* gene.

ACKNOWLEDGMENTS

We thank families A, B1, and B2 for their helpful cooperation, Pr. B.G. Forget for communicating intronic sequences prior to publication [1], Dr. D. Dhermy for providing us with an anti-ankyrin antiserum, Dr. M. Marden for reading the manuscript, Mrs. A. Vallier, Mrs. M.A. Schreiner, and Mr. D. Marmonier for their technical assistance, and Mrs. N. Connan for carefully preparing this manuscript. This work was supported in part by Centre National de la Recherche Scientifique (CNRS URA 1171), Programme Hospitalier de Recherche Clinique (1995), Université Claude-Bernard Lyon-I, Institut National de la Santé et de la Recherche Médicale (CRE 930405), Conseil de la Région Rhône-Alpes, Fondation pour la Recherche Médicale, Association Française contre les Myopathies, Association de Recherche contre le Cancer, and Institut Pasteur de Lyon.

REFERENCES

1. Gallagher PG, Tse WT, Scarpa AL, Lux SE, Forget BG: Structure and organisation of the human ankyrin-1 gene. Basis for complexity of the pre-mRNA processing. J Biol Chem 272:19220, 1997.
2. Peters LL, Lux SE: Ankyrins: Structure and function in normal cells and hereditary spherocytes. Semin Hematol 30:85, 1993.
3. Lux SE, Tse WT, Menninger JC, John KM, Harris P, Shalev O, Chilcote RR, Marchesi SL, Watkins PC, Bennett V, McIntosh S, Collins FS, Francke U, Ward DC, Forget BG: Hereditary spherocytosis associated with deletion of human erythrocyte ankyrin gene on chromosome 8. Nature 345:736, 1990.
4. Eber SW, Gonzalez JM, Lux ML, Scarpa AL, Tse WT, Dornwell M, Herbers J, Kugler W, Ozcan R, Pekrun A, Gallagher PG, Schröter W, Forget BG, Lux SE: Ankyrin-1 mutations are a major cause of dominant and recessive hereditary spherocytosis. Nature Genet 13:214, 1996.
5. Miraglia del Giudice E, Hayette S, Bozon M, Perrotta S, Alloisio N, Vallier A, Iolascon A, Delaunay J, Morlé L: Ankyrin Napoli: A de novo deletional frameshift mutation in exon 16 of ankyrin gene (ANK1) associated with spherocytosis. Br J Haematol 93:828, 1996.
6. Özcan R, Jarolim P, Brabec V, Lux SE, Eber SW: High frequency of frameshift/nonsense mutations of ankyrin-1 in Czech patients with dominant hereditary spherocytosis (DHS) (Abstr). Blood 88:5a, 1996.
7. Gallagher PG, Ferreira JDS, Saad STO, Kerbally J, Costa FF, Forget B: A recurring frameshift mutation of the ankyrin-1 gene associated with severe hereditary spherocytosis in Brazil (Abstr). Blood 88:6a, 1996.
8. Morlé L, Bozon M, Alloisio N, Vallier A, Hayette S, Pascal O, Monier D, Philippe N, Forget BG, Delaunay J: Ankyrin Bugey: A de novo deletional frameshift variant in exon 6 of ankyrin gene (ANK1), associated with spherocytosis. Am J Hematol 54:242, 1997.
9. Randon J, Miraglia del Giudice E, Bozon M, Perrotta S, De Vivo M, Iolascon A, Delaunay J, Morlé L: Frequent de novo mutations of the ANK1 gene mimic a recessive mode of transmission in hereditary spherocytosis. Three new ANK1 variants: ankyrin Bari, Napoli II and Anzio. Br J Haematol 96:500, 1997.
10. Lux SE, Palek J: Disorders of the red cell membrane. In Handin RI, Lux SE, Stossel TP (eds): ''Blood, Principles and Practice of Hematology.'' Philadelphia: Lippincott, 1995, pp 1701–1818.
11. Jarolim P, Rubin HL, Brabec V, Palek J: Comparison of the ankyrin $(AC)_n$ microsatellites in genomic DNA and mRNA reveals absence of one ankyrin mRNA allele in 20% of patients with hereditary spherocytosis. Blood 85:3278, 1995.
12. Agre P, Orringer EP, Bennett V: Deficient red-cell spectrin in severe, recessively inherited spherocytosis. N Engl J Med 306:1155, 1982.
13. Jarolim P, Brabec V, Lambert S, Liu SC, Zheu Z, Palek J: Ankyrin Prague: A dominantly inherited mutation of the regulatory domain of ankyrin associated with hereditary spherocytosis (Abstr). Blood 76: 37a, 1990.
14. Jarolim P, Rubin HL, Brabec V, Palek J: Abnormal alternative splicing of erythroid ankyrin mRNA in two kindred with hereditary spherocytosis (Ankyrin^{Prague} and ankyrin^{Rakovnik}) (Abstr). Blood 82:5a, 1993.
15. Reman O, Carré G, Belloy F, Galateau F, Cheze S, Macro M, Leporrier M: Extramedullary haematopoiesis in hereditary spherocytosis simulating mediastinal tumour. Eur J Haematol 58:124, 1997.
16. Fairbanks G, Steck TL, Wallach DFH: Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606, 1971.
17. Delaunay J: Disorders of the erythrocyte membrane skeleton. In Davis S (ed): ''Critical Reviews in Oncology/Hematology.'' Ireland Ltd.: Elsevier Scientific Publishers, 1995, 19, pp 79–110.
18. Jarolim P, Rubin HL, Brabec V, Palek J: A nonsense mutation 1669 Glu→Ter within the regulatory domain of human erythroid ankyrin leads to a selective deficiency of the major ankyrin isoform (Band 2.1) and a phenotype of autosomal dominant hereditary spherocytosis. J Clin Invest 95:941, 1995.
19. Cooper DN: Human gene mutations affecting RNA processing and translation. Ann Med 25:11, 1993.
20. Dietz HC, Valle D, Francomano CA, Kendzior RJ Jr, Pyeritz RE, Cutting GR: The skipping of constitutive exons in vivo induced by nonsense mutations. Science 259:680, 1993.
21. Belgrader P, Maquat LE: Nonsense but not missense mutations can decrease the abundance of nuclear mRNA for the mouse major urinary protein, while both types of mutations can facilitate exon skipping. Mol Cell Biol 14:6326, 1994.
22. Hall TG, Bennett V: Regulatory domains of erythrocyte ankyrin. J Biol Chem 262:10537, 1987.
23. Davis LH, Davis JQ, Bennett V: Ankyrin regulation: An alternatively spliced segment of the regulatory domain functions as an intramolecular modulator. J Biol Chem 267:18966, 1992.